Accepted for publication 29 October 2009. Published 25 January 2010.

Pythium Root Dysfunction of Creeping Bentgrass

James P. Kerns, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706; and **Lane P. Tredway,** Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695

Corresponding author: James Kerns. jpk@plantpath.wisc.edu

Kerns, J. P., and Tredway, L. P. 2010. Pythium root dysfunction of creeping bentgrass. Online. Plant Health Progress doi:10.1094/PHP-2010-0125-01-DG.

Disease: Pythium root dysfunction (syn. = Pythium-induced root dysfunction).
Primary Hosts: Creeping bentgrass (*Agrostis stolonifera* L.)
Pathogens: *Pythium volutum*, Vanterp. & Truscott (1932); *P. aristosporum*, Vanterp. (1938); and *P. arrhenomanes*, Drechsler (1928).

Taxonomy

Pythium volutum Vanterp. & Truscott, *P. aristosporum* Vanterp., and *P. arrhenomanes* Drechsler were first recognized as new species within the genus *Pythium* in 1932, 1938, and 1928, respectively. *P. aristosporum* and *P. arrhenomanes* were documented as causal agents of PRD in 1985 by Hodges and Coleman (4). *P. volutum* was first documented as a pathogen of creeping bentgrass in 1994 by Abad et al. (1) and as a causal agent of Pythium root dysfunction (PRD) by Kerns and Tredway in 2007 (6).

Symptoms and Signs

Pythium root dysfunction is a disease of relatively young stands (≤ 8 years old) of creeping bentgrass (*Agrostis stolonifera* L.) turf (3,4,7). Symptoms are most severe in high sand-content rootzones with excellent infiltration and percolation (3,4,7). Symptoms of PRD typically appear during the summer months when creeping bentgrass is subjected to heat and drought stress, however patches may develop in the spring, fall, and winter during periods of warm and dry weather. Initially, symptoms appear as circular areas of wilt, chlorosis or drought stress that range in size from 4 to 16 cm in diameter (Fig. 1). These areas progress to larger, irregular patches of yellow, orange foliar decline ranging in size from 16 to 50 cm (Fig. 2). Eventually, if the affected areas are left untreated, large areas of turf can be killed (Fig. 3). Root depth within affected areas may be significantly reduced when compared to unaffected turf areas (Fig. 4). Affected creeping bentgrass roots have bulbous root tips, lack root hairs, and have a light tan color (Fig. 5).

Fig. 1. Initial symptoms of Pythium root dysfunction on a creeping bentgrass putting green in North Carolina. Symptoms typically appear as small circular areas of wilt or drought stress and can appear during any season.

Fig. 2. Progression of Pythium root dysfunction symptoms to larger, more irregular patches on a creeping bentgrass putting green. Note how the stand symptoms have a more yellow-orange appearance when compared to the initial symptoms.

Fig. 3. If left untreated, Pythium root dysfunction can progress to kill large areas of creeping bentgrass. Note the similarity of these symptoms take-all patch.

Fig. 4. Comparison of rooting depth between a healthy creeping bentgrass area (right profile) and a creeping bentgrass area experiencing symptoms of PRD (left profile). Significant reductions in rooting depth are typically only observed during the summer months.

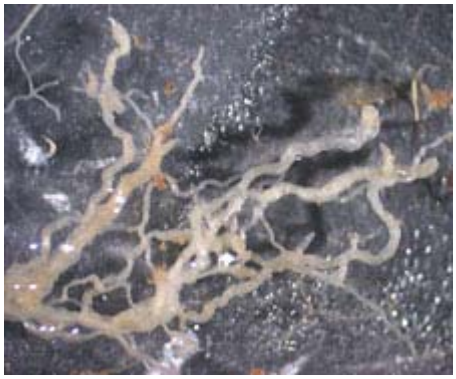

Fig. 5. Effect of *Pythium volutum* infections on creeping bentgrass roots. Although the roots look healthy, they have a slight tan color, have bulbous root tips, and lack root hairs.

Although PRD symptom development is most common in summer months, infection occurs at lower soil temperatures. *P. volutum* infects creeping bentgrass roots when soil temperatures are between 12 and 24°C, without inducing foliar symptoms (7,8). Hodges and Coleman (4) observed that extensive root colonization occurred when *P. aristosporum* and *P. arrhenomanes* coexisted at temperatures ranging from 18 to 26°C. Yet, they suggested that under optimal growing conditions the host and pathogen may coexist without evidence of the disease. Feng and Dernoeden (3) demonstrated that *P. volutum* was more aggressive towards creeping bentgrass seedlings at 18°C than at 28°C, yet *P. aristosporum* was highly aggressive at 18 and 28°C. Feng and Dernoeden (3) did note that 77% of the 28 isolates they collected were obtained during relatively cool, moist periods.

During these periods of pathogen activity, oospores and coenocyctic hyphae are readily observed in cortical cells of creeping bentgrass roots (Fig. 6). Frequently bisporous oospores are observed along the vascular cylinder of affected roots (Fig. 7). The number of oospores present in the root tissue does not seem to be a factor in the amount of disease that is observed. Oospore number can be low, yet the potential for root disease still exists. Although a more efficient diagnostic technique is needed, the best means for diagnosis of PRD at this time is observation of characteristic plant and stand symptoms combined with microscopic confirmation of the pathogen's presence.

Fig. 6. The majority of *P. volutum* oospores develop in the cortical tissue of creeping bentgrass roots. Oospore production is most prolific during the fall and spring.

Fig. 7. Oospores produced by *P. volutum* in creeping bentgrass roots are commonly bisporous. *Pythium volutum* will also produce bisporus oospores in grass-leaf cultures, but less frequently than observed *in vivo*. (Photo by P. H. Dernoeden, © American Phytopathological Society).

Another factor that complicates PRD diagnosis is that foliar symptoms commonly do not appear until the turf is subjected to heat and drought stress. The disease becomes very difficult to diagnose at this time, as heavily infected roots die when subjected to heat and are easily detached from symptomatic plants. When oospores and hyphae characteristic of *Pythium* species are not present, the disease can be tentatively identified based on symptoms observed on the turf stand and roots as discussed previously (3).

Fig. 8. Individual patch of Pythium root dysfunction on a creeping bentgrass putting green. The profiles in the picture represent a diseased area and a non-diseased area. Note how the sand clings to the non-diseased roots and not the diseased roots.

Symptoms typically appear first on mounds and along slopes, as these are areas that are most prone to drought stress. Pythium root dysfunction symptoms are generally most severe in putting greens that have good air circulation and with full sun. Many times golf course superintendents will report symptoms characteristic of PRD after a major club tournament, especially if the greens were mowed lower or allowed to dry out during the event. Root depth may not be affected within the patch early in the season, but significant reductions in root depth are typically observed in mid- to late-summer as infected roots die-back more rapidly than uninfected roots upon heat exposure. Regardless of their depth, roots infected by the PRD pathogens typically lack root hairs, which is a useful diagnostic feature. In the field, sand will not cling to roots affected by *P. volutum* as it does to healthy roots, presumably due to the lack of root hairs (Fig. 8).

Pythium root dysfunction is commonly misdiagnosed as take-all patch, caused by the ectotrophic root-infecting fungus *Gaeumannomyces graminis* var. *avenae*. This confusion results from the similar stand symptoms induced by these two diseases, a mild necrosis of the crown induced by PRD that resembles take-all patch (Fig. 8), and the aforementioned difficulties in observing the dormant PRD pathogen during summer. Diagnosticians should handle suspected samples of PRD carefully to avoid detaching the dead and dying roots that may be infected with Pythium species. Diagnoses of take-all patch should also be confirmed by observation of darkened vascular cylinders (Fig. 10) and dark runner hyphae producing simple hyphopodia (Fig. 11).

Fig. 9. Necrotic crowns associated with creeping bentgrass plants suffering from PRD. This plant symptom is usually only observed during the summer months. This symptom commonly leads to a misdiagnosis of take-all patch, yet copious amounts of runner hyphae typical of take-all patch are not observed in the necrotic areas.

In the Southeast region of the United States, *P. volutum* is the predominate pathogen inducing Pythium root dysfunction on creeping bentgrass putting greens (7). *P. arrhenomanes* and *P. aristosporum* were documented as the major pathogens inducing Pythium root dysfunction in Iowa and the Mid-Atlantic region (3). Although different species were implicated as the causal agents of PRD, symptoms reported in Iowa and the Mid-Atlantic region were similar to those observed throughout the Southeast.

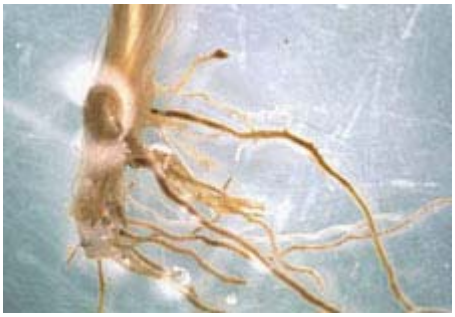

Fig. 10. Necrotic vascular cylinders and dark runner hyphae associated with take-all patch of creeping bentgrass.

Fig. 11. Simple hyphopodia characteristic of *Gaeumanomyces gramins* var. *avenae* on creeping bentgrass roots. (Photo by P. J. Landschoot, © American Phytopathological Society).

Host Range

P. volutum, *P. aristosporum*, and *P. arrhenomanes* have very wide host ranges and have been documented as pathogens of a variety of agronomic, horticultural, and ornamental plants. However, in the turfgrass industry, these pathogens are only known to infect creeping bentgrass grown on golf course putting greens. It is not known if these species can induce a similar disease in annual bluegrass or related bentgrass species, such as colonial (*Agrostis capillaries*) or velvet bentgrasses (*Agrostis canina*). All bentgrass cultivars commonly planted in the Southeast are susceptible to infection by *P. volutum*, however varying levels of resistance or tolerance have been observed among cultivars (9). The relative resistance of creeping bentgrass cultivars to *P. aristosporum* and *P. arrhenomanes* is unknown.

Geographic Distribution

Pythium root dysfunction was first reported and described as a disease of creeping bentgrass on golf course putting greens in Iowa in 1985 (4). However, the disease has been observed throughout the Upper Midwest and Canada since 1977 (4). Pythium root dysfunction has been found throughout the Mid-Atlantic and Southeastern United States as well (3,7). Pythium root dysfunction is likely a problem of creeping bentgrass throughout the eastern half of the United States.

Pathogen Isolation

Depending on the time of year, isolation of *P. volutum* from creeping bentgrass root tissue can be difficult. During mid to late summer, when the bulk of symptom development occurs, *P. volutum* is very difficult to isolate. Isolation from infected root tissue may be accomplished by washing 3 to 5-mm sections of symptomatic roots in continuously flowing tap water for at least 6 h. Root sections should then be blotted dry on sterile paper towels, plated on clarified V-8 juice agar (SV8) (7,11), and incubated in the dark at 18 to 22°C. Colonies free from bacterial and fungal contaminants should be transferred to fresh SV8 agar. If this method is not successful, a previously described baiting method may be used (7). Baiting can be accomplished by placing washed root sections in the root zone of 'A-4' creeping bentgrass seedlings grown in calcined clay (Turface Allsport, Profile Products LLC, Buffalo Grove, IL), incubating in a saturated condition at room temperature for 5 to 7 days, washing the infected seedling roots as described above, and plating on SV8.

Hodges and Coleman (4) did not report their isolation method, however Feng and Dernoeden (3) employed a different baiting technique. They placed infected roots into a Petri dish containing 12 pieces of sterile annual bluegrass leaves (0.5 cm to 1.0 cm long) and sterile deionized water. The infected leaves were blotted dry and placed on water agar. Cultures were incubated for 48 to 96 h. Feng and Dernoeden (3) also plated infected roots on PARP media and incubated the cultures at 25°C. Species of *Pythium* react differently to the typical antibiotics (ampicillin and pimaricin) used in *Pythium* specific media. *P. volutum* growth in particular is suppressed by some of these antibiotics, which may be the reason why Abad et al. (1) and Feng and Dernoeden did not isolate *P. volutum* frequently (7). It is recommended that researchers and diagnosticians employ one of the baiting techniques mentioned above in addition to using a selective medium in order to prevent inadvertently selecting for or against certain *Pythium* species.

Pathogen Identification

Identification of *Pythium* species is based on the morphology and dimensions of oospores, oogonia, antheridia, sporangia, and hyphae. The production of these structures can be induced in grass-leaf water cultures (Fig. 12) (1,7,11), as most species of *Pythium* that infect turfgrasses are homothallic. Briefly, grass-leaf blade cultures consist of 1-cm long autoclaved grass blades (preferably creeping bentgrass or tall fescue) and sterilized DI water, in which three or four agar plugs (#3 or 4 cork borer) infested with the unknown isolate are placed. When incubated under continuous fluorescent light at room temperature, sporangia typically develop within 48 h and oogonia, antheridia, and oospores develop after 48 to 96 h The following morphological descriptions are summarized from the reports of Abad et al. (1), Dick (2), and Kerns and Tredway (7).

Fig. 12. Grass leaf blade water cultures infested with *P. volutum*. Each culture contains approximately 15 to 20 ml of H_2O, 5 to 8, 1 to 2 cm long grass leaf blades and three agar plugs taken from 3-day-old *P. volutum* cultures.

P. volutum. Oogonia range in diameter from 22 to 34 µm with an average of 30 µm and are produced terminally or intercalary. Oospores are large (24 to 38-µm diameter), aplerotic (oospore does not completely fill oogonium) and thick-walled and occasionally bisporus (Fig. 13). Antheridia are mostly diclinous (having an antheridium on one hypha with the oogonium on another) with 3 to 10 per oogonium. Usually, one antheridium coils around the oogonial stalk (Fig. 14). Sporangia are lobate and typically intercalary (Fig. 15). Colonies of *P. volutum* on clarified V8 juice agar are cottony at first and over time the hyphae submerges back to the agar surface (Fig. 16).

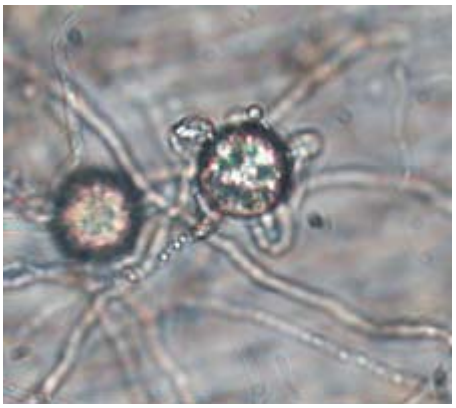

Fig. 13. *Pythium volutum* oogonia surrounded by four antherdia in grass-leaf cultures at 400x magnification. Note that the antheridia are diclinous and somewhat inflated.

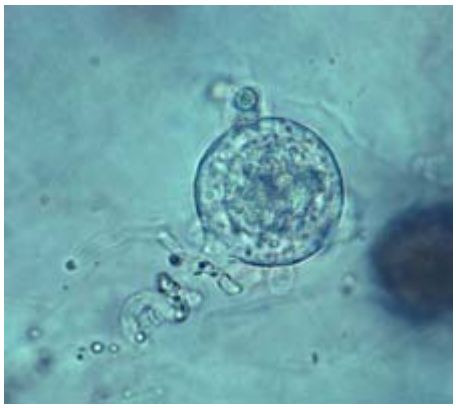

Fig. 14. *Pythium volutum* oogonia in grass-leaf blade culture at 400x magnification showing an antheridium coiling around the oogonial stalk. This is an important diagnostic feature for *P. volutum* because it is one of three *Pythium* species that display this trait.

Fig. 15. Example of an intercalary lobate sporangia of *P. volutum*. *Pythium volutum* is not a prolific producer of sporangia in culture, therefore identification should focus on oogonial, antheridial, and oospore morphology.

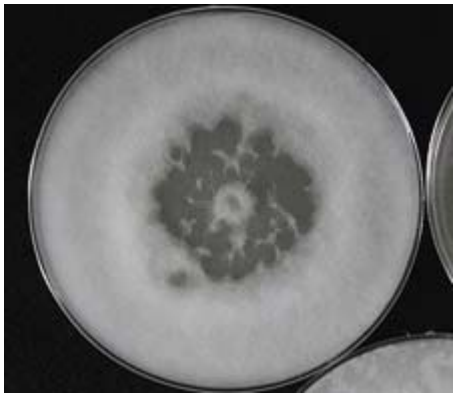

Fig. 16. Colony morphology of *P. volutum* grown on clarified V8 juice agar. Colony is 7 days old and has white, fluffy aerial mycelium that has started to submerge back to the medium surface.

P. arrhenomanes. Sporangia are large and lobate (Fig. 17). The sexual stage for *P. arrhenomanes* is very scarce even in grass-blade cultures. However, if induced, antheridia are diclinous and abundant (up to 25 per oogonia) with crooked-necks. Oogonia are mostly terminal and range in diameter from 24 to 36 µm (average 32.5 µm) (Fig. 18). Oospores are plerotic and commonly degenerate or abortive (> 50%) (Fig. 19). Colonies of *P. arrhenomanes* are radiate or submerged depending on the growth medium.

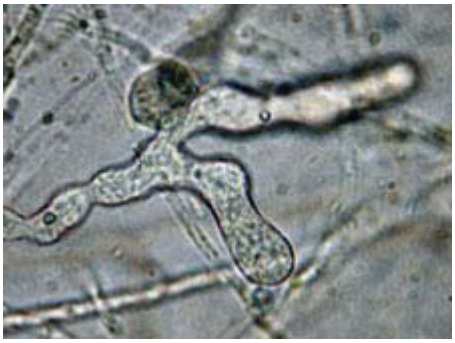

Fig. 17. Filamentous, inflated, lobate sporangia of *P. arrhenomanes* produced in grass-leaf blade cultures. (Courtesy of Gloria Abad, USDA).

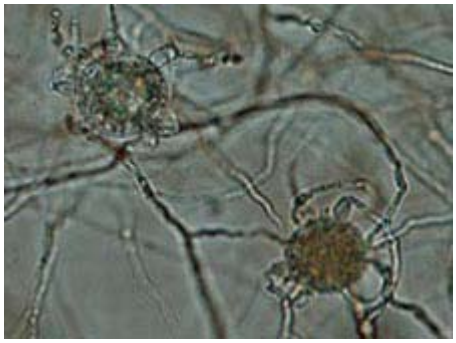

Fig. 18. *P. arrhenomanes* oogonia and antheridia produced in grass-leaf blade culture. Production of antheridia is rare in culture. The oogonia are mostly terminal and range in diameter from 24 to 36 µm. Antheridia are numerous (up to 25 per oogonia) and have crooked-necks. (Courtesy of Gloria Abad, USDA).

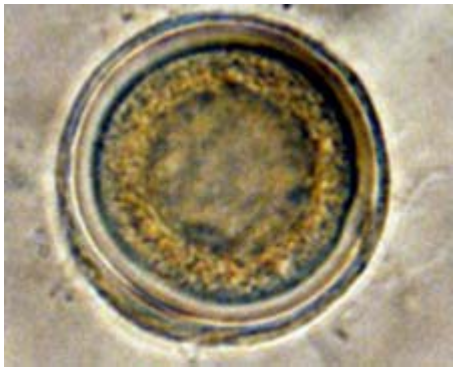

Fig. 19. *P. arrhenomanes* oospores produced in grass-leaf blade cultures. Typical oospores are plerotic and are frequently degenerate or abortive. (Courtesy of Gloria Abad, USDA).

P. aristosporum. Isolates have monoclinous (having an antheridium on the same small branch or pedicel that bears the oogonium) antheridia that are club-shaped or crook-necked with up to 8 per oogonium (Fig. 20). Oogonia are mainly terminal with diameters ranging in size from 27 to 45 µm (average 32.7 µm). Oospores are aplerotic and large (24 to 44 µm diameter) (Fig. 21). Sporangia are lobate and inflated, but smaller than sporangia of *P. arrhenomanes* (Fig. 22). Colony morphology is dependant on the type of growth medium, but is typically radiate or submerged.

Fig. 20. Oogonia and antheridia of *P. aristosporum* produced in grass-leaf blade cultures. Antheridia are monoclinous, are typically club-shaped or crook-necked with up to 8 per oogonium. Oogonia range in size from 27 to 45 µm with an average diameter of 32.7 µm. (Courtesy of Gloria Abad, USDA).

Fig. 21. Oospore of *P. aristosporum* produced in grass-leaf blade cultures. Oospores are aplerotic and large, with diameters ranging from 24 to 44 µm in diameter. (Courtesy of Gloria Abad, USDA).

Fig. 22. Sporangia production of *P. aristosporum* in grass-leaf blade cultures. Sporangia are lobate and filamentous to toruloid.

Species-specific PCR primers have been developed for various *Pythium* species, but they cannot differentiate between these three species (10). However, amplification and sequencing of rDNA regions with the universal primers ITS 4 and ITS5 is fairly simple and an effective tool to support morphological identifications (7).

Pathogen Storage

Isolates of *P. volutum* have been stored for up to 6 months in water blanks incubated at 18°C without significant loss of viability. Briefly, agar plugs are removed from 3 or 4 d old colonies of *P. volutum* and placed in sterile glass or plastic test tubes containing 20 ml sterilized DI water. Placing agar plugs into fresh grass blade cultures can revive stored isolates, and then infested grass

blades can be plated onto fresh SV8 media. Storage of *P. aristosporum* and *P. arrhenomanes* can also be accomplished using the technique mentioned above.

Pathogenicity Tests

Pythium root dysfunction is a disease of secondary creeping bentgrass roots, therefore it is recommended that seedlings not be used as a measure of pathogenicity. Inoculation of creeping bentgrass roots with grass-leaf blade cultures of *P. volutum* has been very successful in growth chamber experiments and moderately successful in field plots. Inoculum is prepared by placing 10 to 15 ml of sterile DI H_2O in a Petri dish containing 5 to 7, 1-cm long autoclaved creeping bentgrass leaves. Agar plugs from 3 to 4-day-old *P. volutum* cultures grown on SV8 are then transferred to the grass-blade water mixture. Cultures should be sealed with Parafilm and incubated at room temperature in continuous fluorescent light for 3 to 4 days. For growth chambers experiments, plants are grown in the greenhouse for at least six weeks prior to inoculation. Because *P. volutum* can cause foliar blight, it is imperative to place the pathogen in the rootzone in order for the pathogen to infect the roots. Inoculations can be accomplished by cutting the root systems at 5 cm and placing grass leaf-blade cultures of *P. volutum* onto the surface of fresh soil medium and then replace the turf plug (7,8,9).

Inoculated plants should be incubated at temperatures between 16 and 24°C for three to four weeks with a 12-h photoperiod to facilitate root infection and colonization. Following this infection stage, inoculated plants are subjected to a heat stress treatment of at least 32°C during the day and 26°C at night to induce expression of foliar symptoms. Inoculated plants should be watered three to four times per week during the heat stress period. No information exists about the influence of day lengths or light intensity on symptom expression. Typically, foliar dieback is observed ten to fourteen days after plants are subjected to heat stress.

Successful inoculation of two to three leaf stage creeping bentgrass plants was achieved by dipping roots in a slurry of mycelium and zoospores (4). The inoculum for this technique was prepared by transferring *P. aristosporum* and *P. arrhenomanes* from corn meal agar to a barley seed medium (5 g of barley seed in 150 ml of distilled water, autoclaved). Cultures were macerated in 100 ml water in a blender after 4 weeks of growth. Then roots of creeping bentgrass were dipped into the slurry. The inoculated plants were then transplanted to pots containing sand or soil medium and were grown in the greenhouse for 8 weeks at 18 to 26°C.

Disease severity can be assed visually. Digital imaging methods for assessment of PRD in growth chamber conditions or the field have been tested. Dry weights of roots and shoots have been shown as an effective assessment of PRD severity in growth chamber conditions (4,7).

Field inoculations can be attempted by removing a golf course cup cutter plug (10-cm diameter) of turf and cutting the roots at 5 cm. The remaining soil in the hole is leveled and grass-leaf inoculum is poured onto the existing soil before replacing the turf plug. Inoculated plots should be watered immediately with no more than 31 mm (1/8 inch) of water and should be kept moist to a depth of 5 cm for 4 or 5 days. Inoculations should be done during the spring when soil temperatures are between 12 and 24°C. Symptoms will not develop until the inoculated plants are subjected to heat and drought stress, usually two to three months after inoculation.

Acknowledgements

We would like to thank Dr. Gloria Abad for providing the excellent photos of *Pythium arrhenomanes* and *Pythium aristosporum*.

Literature Cited

1. Abad, Z. G., Shew, H. D., and Lucas, L. T. 1994. Characterization and pathogenicity of *Pythium* species isolated from turfgrass with symptoms of root and crown rot in North Carolina. Phytopathology 84:913-921.
2. Dick, M. W. 1990. Keys to *Pythium*. Coll. of Estate Manag., Whiteknights, Reading,UK.
3. Feng, Y., and Dernoeden, P. H. 1999. *Pythium* species associated with root dysfunction of creeping bentgrass in Maryland. Plant Dis. 83:516-520.
4. Hodges, C. F., and Coleman, L. W. 1985. *Pythium*-induced root dysfunction of secondary roots of *Agrostis palustris*. Plant Dis. 69:336-340.
5. Hodges, C. F., and Campbell, D. A. 1994. Infection of adventitious roots of *Agrostis palustris* by *Pythium* species at different temperature regimes. Can. J. Bot. 72:378-383.
6. Kerns, J. P., and Tredway, L. P. 2007. First report of Pythium root dysfunction of creeping bentgrass caused by *Pythium volutum* in North Carolina. Plant Dis. 91:632.
7. Kerns, J. P., and Tredway, L. P. 2008. Pathogenicity of *Pythium* species associated with Pythium root dysfunction of creeping bentgrass and their impact on root growth and survival. Plant Dis. 92:862-869.
8. Kerns, J. P., and Tredway, L. P. 2008. Influence of temperature on pathogenicity of *Pythium volutum* toward creeping bentgrass. Plant Dis. 92:1669-1673.
9. Kerns, J. P., Shew, H. D., Benson, D. M., and Tredway, L. P. 2009. Impact of irrigation frequency, organic matter content and creeping bentgrass cultivar on the development of Pythium root dysfunction. Int'l. Turfgr. Soc. Res. J. 11:227-239.
10. LeVesque, C. A., and De Cock, A. W. A. M. 2004. Molecular phylogeny and taxonomy of the genus *Pythium*. Mycological Res. 108:1368-1383.
11. Martin, F. N. 1992. *Pythium*. Pages 39-49 in: Methods for research on soilborne phytopathogenic fungi. L. L. Singleton, J. D. Mihail, and C. M. Rush, eds. American Phytopathological Society, St. Paul, MN.